

P201**Chondrotoxic effects of local anesthetics used in peri-operative pain pumps**

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Purpose: Chondrolysis initiated by post-operative, intra-articular pain pumps has recently been described by multiple institutions. This study evaluates the chondrotoxicity of anesthetic formulations commonly used in pain pumps.

Methods and Materials: Freshly isolated human articular chondrocytes were cultured in a custom bioreactor, which mimics metabolism of synovial fluid and medication, for 24, 48, and 72 hour trials. Chondrocytes were perfused in DMEM-10% FBS and one of the following medications: 1% Lidocaine, 1% Lidocaine with epinephrine, Bupivacaine 0.25%, Bupivacaine 0.25% with epinephrine, Bupivacaine 0.5%, Bupivacaine 0.5% with epinephrine. Static and perfusion cultures with growth media were used as controls. All experiments were run in duplicate. Live/Dead staining was performed and the ratio of dead:live cells were assessed by fluorescent microscopy.

Results: Significantly more chondrocyte necrosis was found in cultures containing 1% Lidocaine, 1% Lidocaine with epinephrine, Bupivacaine 0.25% with epinephrine, Bupivacaine 0.5% with epinephrine compared to controls ($p < 0.05$) at 48 and 72 hours. 0.25% and 0.05% Bupivacaine exhibited no cytotoxic effects at any time point.

Conclusions: 0.25% and 0.5% Bupivacaine do not cause chondrocyte necrosis and are likely to be safe when used in pain pumps for 72 hours. All medications containing epinephrine ($pH < 4$) were chondrotoxic and cannot be advocated for pain pump use.

P202**Time-lapse confocal imaging of living chondrocytes in appositionally developing outgrowths at the agarose-media interface**N. Tran-Khanh¹, M. Lavertu¹, V. Lascau-Coman², C.D. Hoemann², M.D. Buschmann³;

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Purpose: Chondrocytes cultured in agarose can develop outgrowths resembling cartilage nodules at the agarose-media interface. The purpose of this study was to design a live cell time-lapse confocal imaging technique to observe the development of hyaline-like outgrowths at the agarose-media interface.

Methods and Materials: Young adult bovine articular chondrocytes were enzymatically released, encapsulated in 2% low melting temperature agarose, and cultured for up to 1 month with daily sodium L-ascorbate supplementation. Cell proliferation in the interfacial outgrowth was observed on fixed cryosections by detection of Ki67 and incorporated BrdU. PKH26 membrane stain and acridine orange (AO) at 10 ng/mL, were used to perform live cell imaging with a confocal microscope equipped with a humidified culture chamber at 37°C.

Results: Ki67 and BrdU staining showed proliferation mainly at the periphery of the outgrowths. PKH26 staining of living cells was weaker in peripheral compared to central cells, showing dye dilution caused by higher cell division in the outer regions. PKH26 time-lapse imaging was not possible because of photobleaching. Continuous incubation with AO allowed the acquisition of hundreds of 3D scans, in the absence of any apparent cellular damage. With the latter technique, we observed time lapse live cell division at the periphery of the outgrowths, as well as several unexpected dynamic features including cell cluster movement and chondrocyte migration over relatively large distances.

Conclusions: Interfacial chondrocyte outgrowths display an appositional growth mechanism partly resembling the growth plate. Time lapse live confocal microscopy of these outgrowths using low AO concentrations provides a new tool to study chondrocyte dynamics and cartilage growth processes.

P203**Phenotypic characterization and redifferentiation of human articular chondrocytes expanded on microcarriers**K. Schrobback¹, T. Klein¹, M. Schütz², D. Hutmacher³, D. Leavesley¹, Z. Upton¹, J. Malda¹;

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Purpose: Microcarrier-based cell culture holds promise for controllable and cost-efficient in vitro expansion of primary articular chondrocytes while promoting the chondrocyte phenotype. Improved understanding of how microcarriers affect chondrogenic differentiation will facilitate incorporation of this technology in future clinical approaches.

Methods and Materials: Human chondrocytes from normal articular cartilage (grade 0-1) were isolated and expanded in monolayers and on Cultispher-G microcarriers. Proliferation was analyzed by DNA quantitation while cell viability was monitored by vital staining with propidium iodide / fluorescein diacetate and MTT. The differentiation potential of expanded cells was evaluated in pellet culture. Glycosaminoglycan (GAG) accumulation was quantified with dimethylmethylene blue and collagen deposition was measured by [³H]-proline incorporation. Histology and immunohistochemistry were also performed. Microcarriers were characterized by micro-computed tomography.

Results: Primary human chondrocytes expanded on microcarriers 10.2±2.6 fold in 3 weeks. Cell viability was >90% at each time point. GAG content significantly increased with time; however, the majority of GAG was found in the medium. Collagen production per ng DNA increased only marginally during expansion. Histological characterization of chondrocytes on the microcarriers revealed that the cells were randomly distributed on the microcarrier surfaces. In contrast most pores were cell free. Micro-computed tomography imaging of the microcarriers confirmed limited interconnectivity of the pores. Critically, human chondrocytes expanded on microcarriers maintained their ability to redifferentiate in pellet culture.

Conclusions: These data confirm the feasibility of microcarriers for the cultivation of human articular chondrocytes and contribute to the development of a simple, passage-free cell culture method for cartilage tissue engineering.

P204**Cytochrome oxidase levels in chondrocytes during expansion and after return to 3-D culture**O. Boubriak¹, J. Urban²;

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Purpose: Articular chondrocytes, though using oxygen at substantial rates, function in situ at <7.5% oxygen and produce energy mainly by glycolysis. During the expansion in monolayer for purposes of autologous chondrocyte implantation or tissue engineering research, monolayer cells dedifferentiate. Here we investigate whether oxygen utilisation is altered by activation of enzymes of the Krebs cycle and electron transport chain during monolayer culture and whether these metabolic pathways are restored on return to 3D culture.

Methods and Materials: Primary bovine articular chondrocytes were cultured either continuously in 3D gels (alginate) or in monolayer to confluence. Cells were stained for cytochrome oxidase (COX) on a daily basis. COX and citrate synthase (SC) activity and mitochondrial protein cell content were measured biochemically. At confluence, cells were harvested and suspended in alginate beads to provide 3D support. 1-4 weeks after return to 3D culture, chondrocytes were stained for COX activity.

Results: Freshly isolated chondrocytes or chondrocytes grown continuously in alginate demonstrated minimal COX staining. After 7 days monolayer culture, almost all cells stained strongly for COX. Mitochondrial protein, COX and SC activity increased noticeably over the 10-day expansion period. COX staining in cells returned to 3D culture remained high for up to 4 weeks if the cells had been cultured for longer than 3-4 days in monolayer.

Conclusions: Chondrocytes expended in monolayer for longer than 3-4 days retained abnormal metabolic enzyme activity even after 4 weeks in 3D culture. The implications for tissue engineering or clinical treatment are at present unclear.